

I/WE CLAIM:

1. A method for detecting a target nucleic acid comprising:

(a) contacting said nucleic acid in said sample in a reaction vessel under conditions that allow hybridization between complementary sequences in nucleic acids with oligonucleotide probe said oligonucleotide probe further comprising 3' and 5' regions that are complementary to adjacent sequences in the target nucleic acid;

(b) ligating the 3' and 5' ends of oligonucleotide with a ligating agent that joins nucleotide sequences such that a circular probe is formed;

(c) adding a oligonucleotide primer pair wherein the first primer of the pair comprises a first sequence that is complementary to the circular probe and serves as a primer for RAM mediated amplification, a second sequence which is complementary to the second primer of the pair, and a signal generating moiety and wherein the second primer of the pair comprises a sequence that is complementary to the first primer and a moiety capable of quenching, masking or inhibiting the activity of the signal generating moiety when located adjacent to or in close proximity to said signal; wherein the primers are designed in such a way that the when the first primer and second primer are bound to one another the signal generating moiety and the quenching, masking or inhibitory moiety are adjacent to, or in close proximity to one another the signal is inhibited;

- (d) amplification of the circular probe resulting in spatial separation of the signal generating moiety from the quenching, masking or inhibitory moiety thereby permitting the detection of signal, wherein detection thereof indicates the presence of the target nucleic acid in the sample.
2. The method of claim 1 wherein the signal generating moiety is a fluorescent agent.
 3. The method of claim 1 wherein the signal generating moiety is a chemiluminescent reagent.
 4. The method of claim 1 wherein the signal generating moiety is an enzyme reagent.
 5. The method of claim 1 wherein the amplification method is selected from the group consisting of polymerase chain reaction, SDA, or TMA
 6. A method for detecting the presence of a target nucleic acid in a sample comprising:
 - (a) contacting said nucleic acid with a hybridization/C-probe complex wherein said complex comprises:
 - (i) a single stranded oligonucleotide hybridization probe having a region that is complementary to the target nucleic acid and a ligand moiety;
 - (ii) a circular probe comprising ligand binding moieties; wherein said oligonucleotide hybridization probe and circular probe are bound to one another;
 - (b) addition of DNA polymerase and primers that bind to the circular

probe; and

- (c) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid in the sample.

7. The method of claim 6 wherein said the ligand moiety is bound to the 5' or 3' end of the single stranded oligonucleotide hybridization probe.
8. The method of claim 6 wherein the single stranded oligonucleotide hybridization probe contains the ligand.
9. The method of claim 6 wherein said ligand is selected from the group consisting of biotin, digoxigenin, antigens, haptens, antibodies, heavy metal derivatives, and polynucleotides.
10. The method of claim 6 wherein said ligand binding moiety is selected from the group consisting of strepavidin, avidin, anti-digoxigenin antibodies, antibodies, antigens, thio groups and polynucleotides.
11. A method for detecting the presence of a target nucleic acid in a sample comprising:
 - (a) contacting said nucleic acid with a hybridization probe wherein said hybridization probe comprises a single stranded oligonucleotide having (i) a 5' region that is complementary to the target nucleic acid; (ii) a ligand moiety and (iii) a 3' region that is complementary to the circular probe and a circular probe wherein said circular probe comprises (i) a ligand binding moiety and (ii) a region that is

complementary to the hybridization probe;

- (b) extending the hybridization probe by addition of DNA polymerase
- (c) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid in the sample.

12. The method of claim 11 wherein the single stranded oligonucleotide hybridization probe contains the ligand internally.

13. The method of claim 11 wherein said ligand is selected from the group consisting of biotin, digoxigenin, antigens, haptens, antibodies, heavy metal derivatives, and polynucleotides.

14. The method of claim 6 wherein said ligand binding moiety is selected from the group consisting of streptavidin, avidin, anti-digoxigenin antibodies, antibodies, antigens, thio groups and polynucleotides.

15. A method for *in situ* detection of a target nucleic acid comprising the steps of :

- (a) addition of a C-probe comprising a ligand binding moiety and a 3' and 5' r
- (b) addition of target nucleic acid molecule such that a complex formed between the target nucleic acid molecule and the C-probe;
- (c) ligating the 3' and 5' ends of the C-probe with a ligating agent that joins nucleotide sequences such that a circular probe is formed;

(d) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid in the sample.

16. The method of claim 15 wherein said ligand is selected from the group consisting of biotin, antigens, haptens, antibodies, heavy metal derivatives, and polynucleotides.

17. The method of claim 15 wherein said ligand binding moiety is selected from the group consisting of strepavidin, avidin, antibodies, antigens, thio groups and polynucleotides.

18. The method of claim 15 wherein in the circular probe is amplified using RAM.

19. The method of claim 15 wherein the circular probe is amplified using HSAM.

20. The method of claim 15 wherein the circular probe is amplified using primer extension.

21. A method for *in situ* detection of a target nucleic acid comprising the steps of :

(a) addition of a C-probe comprising a ligand binding moiety and a 3' and 5' region that are complementary to sequences in the target nucleic acid molecule, to

a gel matrix comprising a ligand moiety, such that a complex is formed within the matrix between the ligand moiety and ligand binding moiety;

(b) addition of target nucleic acid molecule such that a complex is formed between the target nucleic acid molecule and the C-probe;

(c) ligating the 3' and 5' ends of the C-probe with a ligating agent that joins nucleotide sequences such that a circular probe is formed;

(d) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid in the sample.

22. The method of claim 21 wherein said ligand is selected from the group consisting of biotin, antigens, haptens, antibodies, heavy metal derivatives, and polynucleotides.

23. The method of claim 21 wherein said ligand binding moiety is selected from the group consisting of strepavidin, avidin, antibodies, antigens, thio groups and polynucleotides.

24. The method of claim 21 wherein in the circular probe is amplified using RAM.

25. The method of claim 21 wherein the circular probe is amplified using HSAM.

26. The method of claim 21 wherein the circular probe is amplified using rolling circle amplification.

27. The method of claim 21 wherein the amplification of the circular probe is carried out in the presence of labeled nucleotides.

28. A method for *in situ* detection of a target nucleic acid comprising the steps of :

- (a) fixation of a oligonucleotide probe to a solid support;
- (b) addition of a gel matrix to the solid support;
- (c) addition of a C-probe comprising (i) sequences that are complementary to the oligonucleotide probe; (ii) and a 3' and 5' region that is complementary to sequences in the target nucleic acid molecule, to the gel matrix such that a complex is formed within the matrix between the oligonucleotide probe and the C-probe;
- (d) addition of target nucleic acid molecule such that a complex is formed between the target nucleic acid molecule and the C-probe;
- (e) ligating the 3' and 5' ends of the C-probe with a ligating agent that joins nucleotide sequences such that a circular probe is formed;

(f) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid in the sample.

29. The method of claim 28 wherein in the circular probe is amplified using RAM.

30. The method of claim 28 wherein the circular probe is amplified using HSAM.

31. The method of claim 28 wherein the circular probe is amplified using rolling circle amplification.

32. A method for *in situ* detection of a target polypeptide comprising the steps of :

(a) embedding said polypeptide within a gel matrix'

(b) addition of a binding partner having an affinity for the polypeptide and further comprising a nucleic acid molecule;

(c) amplification of the nucleic acid molecule wherein detection of amplification of the nucleic acid molecule indicates the presence of the target polypeptide.

33. The method of claim 21 wherein nucleic acid molecule is a circular probe..

34. The method of claim 32 wherein in the circular probe is amplified using RAM.

35. The method of claim 32 wherein the circular probe is amplified using HSAM.

36. The method of claim 32 wherein the circular probe is amplified using rolling circle amplification.

37. The method of claim 32 wherein the amplification of the target nucleic acid molecule is carried out in the presence of labeled nucleotides.

38. A method for detection of a target nucleic acid in a sample comprising:

- (a) contacting said nucleic acid with a hybridization probe wherein said hybridization probe comprises a single stranded oligonucleotide having (i) a region that is complementary to the target nucleic acid and (ii) a region complementary to the circular probe;
 - (b) contacting said nucleic acid with a circular probe wherein said circular probe comprises a single stranded oligonucleotide having (i) a region that is complementary to the target nucleic acid and a region complementary to the hybridization probe, wherein said hybridization probe acts as a primer for amplification of the circular probe in the presence of the target nucleic acid;
 - (c) extending the hybridization probe by addition of DNA polymerase;
- and
- (d) amplification of the circular probe wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid.

39. A method for detection of a target nucleic acid in a sample comprising:

(a) contacting said nucleic acid with a first hybridization probe linked to a solid support wherein said hybridization probe comprises a single stranded oligonucleotide having (i) a region that is complementary to the target nucleic acid; and (ii) a circular probe bound by complementary sequences to said second hybridization probe:

wherein in the presence of a target nucleic acid molecule the first hybridization probe and second hybridization probe are adjacent to one another;

(c) ligating the first hybridization probe to the second hybridization probe; and

(d) amplification of the circular probe

wherein detection of amplification of the circular probe indicates the presence of the target nucleic acid molecule.

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